and clinical studies by different specialists in different countries. Successful instances of co-operation of this type are already available.

f Formal and effective recognition by Health Authorities of the results obtained through international co-operation for the approval of new drugs would be of invaluable help in overcoming the present technical difficulties. As a consequence, it would make all co-operative efforts more efficient and productive for drug research, and, ultimately, for the benefit of the patients in all countries.

g To further these aims, the formulation of guidelines in general and for specific classes of drugs is judged to be extremely useful, provided that they are agreed upon on an international basis and allow the necessary flexibility, essential in applied as well as in basic research.

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STUDIES OF THE INTERACTION OF DESMETHYLIMIPRAMINE WITH TYRAMINE IN MAN AFTER A SINGLE ORAL DOSE, AND ITS CORRELATION WITH PLASMA CONCENTRATION

The pharmacological activity of a new tricyclic antidepressant compound is difficult to assess in normal subjects, as the steady-state plasma concentration is usually not reached before 10-14 days medication (Alexanderson & Sjöqvist, 1971). Volunteers are reluctant to take a compound which may possess marked sedative and anticholinergic properties. The mode of action of tricyclic antidepressant drugs is controversial, but the most widely held hypothesis is that they act by inhibiting the reuptake of biogenic amines including noradrenaline at central receptor sites. This increases the functionally active noradrenaline for the synaptic cleft (Schildkraut, 1970).

During the past few years, tyramine, an indirectly acting sympathomimetic amine, has been used as a pharmacological tool to study this reuptake blocking phenomena (Siwers, Tuck, Freyschuss, Azarnoff & Sjöqvist, 1969). Freyschuss, Sjöqvist & Tuck (1970) showed a highly significant correlation ($r = 0.84$) between the tyramine pressor response and the steady-state plasma level of nortriptyline in patients suffering from depressive illness. Desmethylimipramine has also been shown to have a noradrenaline reuptake blocking effect in rats by Gessa, Vargill & Crabai (1966) and in man by Mitchell, Cavanaugh, Arias & Oates (1970).

In the following experiments we have investigated the effects of desmethylimipramine, 3 h after a single oral dose, on the tyramine pressor response and its correlation with the plasma concentration of the drug. As a tyramine pressor test is contraindicated in subjects suffering from cardiovascular and certain neurological disorders, we have also explored an alternative method to study the blocking effect of tricyclic drugs which could be applicable in subjects where large doses of tyramine are contraindicated.

1 *Studies of the pressor response of tyramine*

Six women and four men with no evidence of significant physical and psychiatric illness and who had normal electrocardiographs, were included in the trial. A double-blind crossover design was used alternating desmethylimipramine (50 mg) and placebo, with at least 7 days interval between the two treatments. During the experiment the subject rested on a couch in the supine position. A butterfly needle was inserted in a left forearm vein and kept patent by flushing with normal saline injection (2 ml). The basal blood pressure (BP) was recorded in the right arm by a standard mercury sphygmomanometer. Three hours after the administration of the compound and 30 min after resting, when the BP reading had become steady, tyramine was rapidly injected starting from a dose of 0.5 mg in a total volume of 2 ml of normal saline. The needle and the cannula were then flushed with normal saline (2 ml). The BP was recorded every 30 s for 5 min or more until the BP had returned to the baseline level. Heart rate was recorded every 30 s by a standard limb lead of an

electrocardiogram. The dose of tyramine was increased according to the response, and the procedure repeated until the systolic BP was elevated by 30 mm Hg or more. From the dose response curve the amount of tyramine required to elevate the systolic BP by 30 mm Hg was determined. The tyramine sensitivity or blocking effect was expressed as the ratio of the dose of tyramine required to elevate systolic BP by 30 mm Hg following desmethylimipramine administration to that required when the subject had received placebo. Blood samples were collected in heparinised tubes at 3 h and 4 h after the oral administration of desmethylimipramine. Plasma concentration was estimated by the method of Gifford, Turner & Pare (1975).

Following rapid tyramine injection the BP increased to a maximum within 1-2.5 min and returned to the baseline BP before 5 minutes. Care was taken to avoid tachyphylaxis by increasing the dose of tyramine as quickly as possible.

Statistical analysis was carried out using two-sided non-parametric tests to avoid assuming that the estimated tyramine responses, tyramine sensitivity and plasma concentrations were normally distributed.

Spearman's rank correlation coefficients were computed to study the relationship between age, height, weight, the tyramine responses following placebo and desmethylimipramine, the tyramine sensitivity and the plasma concentrations of desmethylimipramine.

The tyramine responses for men and women were compared by Mann-Witney U tests. A sign test was used to test the differences between the tyramine responses after placebo and desmethylimipramine to see whether desmethylimipramine shifted the tyramine dose-response curve.

A marked positive correlation was found between the tyramine sensitivity and both the 3 h plasma concentration of desmethylimipramine ($r_s + 0.68$, $n = 10$, $P < 0.05$) and the 4 h concentration ($r_s = 0.67$, $n = 8$, $P < 0.06$). Figure 1 illustrates the correlation between the sensitivity and the 3 h plasma concentration. The significance levels of $P < 0.05$ and $P < 0.06$ are probably due to the small sample sizes. In nine out of ten cases the tyramine response following desmethylimipramine was greater than the corresponding response following placebo. In one case the responses were equal. Thus there appeared to be a shift in the tyramine dose-response curve following desmethylimipramine which was significant ($P < 0.004$).

No significant correlations were found between the tyramine pressor responses after placebo or desmethylimipramine and height, weight or age.

On average male volunteers needed more tyramine than female volunteers following both placebo and desmethylimipramine. Following desmethylimipramine the mean dose for men was 18.0 mg and for women was 10.2 mg ($P < 0.05$). Following placebo the mean dose for men was 6.5 mg and for women was 4.7 mg ($P < 0.10$). This sex difference confirms our earlier observations in depressed and normal subjects (Ghose, Turner & Coppen, 1975).

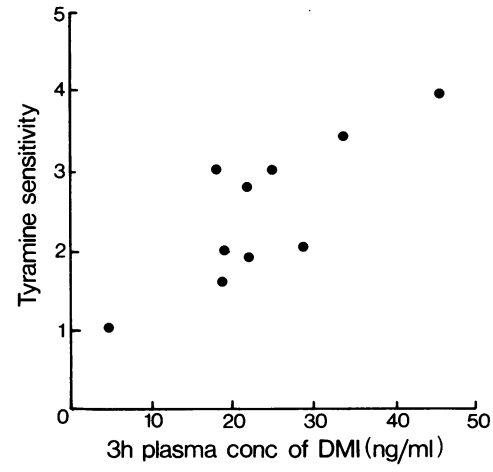

Figure 1 Relationship between tyramine sensitivity (DMI response/placebo response) and the 3 h plasma concentration of desmethylimipramine (DMI) after oral administration of DMI (50 mg) ($r_S = +0.65$, $n = 10$, $P < 0.05$).

2 The measurement of the superficial venoconstrictor effect of tyramine

Hand vein diameter was measured by the modified method of Nachev, Collier & Robinson (1971) described by White & Udwadia (1975), using an electronic displacement transducer.

Six female volunteers (age 20-22 years) participated in the study, which was a double-blind crossover design alternating desmethylimipramine (50 mg) and placebo with at least 7 days interval between the two treatments. The subject rested comfortably on a couch; a superficial vein on the dorsum of the hand was cannulated and kept patent with continuous normal saline infusion at the rate of 0.1 ml/min. The hand and forearm rested on a slanting wooden slope to avoid accumulation of subcutaneous fluid in the hand during the experiment. Hand vein diameter was measured 1 cm proximal to the needle tip by an electronic displacement transducer. The control or baseline record was taken during normal saline

infusion after obliterating the superficial venous flow by inflating a sphygmomanometer cuff around the upper arm. The experiment started when three or more consecutive hand vein diameter readings were identical and 3 h after the administration of the compound. Tyramine was injected slowly starting from a concentration of 1 μg/min. Hand vein diameter was measured at the end of 5 min infusion. The dose of tyramine was then increased according to the previous response. It is impractical to take baseline readings after each concentration, as once the vein is constricted, it takes about 20-25 min to return to the basal state. The experiment was continued until there was no further constriction of the vein with increasing dose, i.e. 100% constriction. From the dose-response curves the tyramine doses required to constrict hand vein diameter by 50% were calculated. Blood samples were collected in heparinised tubes and the plasma concentration was estimated at 3 h and 5 h after desmethylimipramine administration. Throughout this experiment the room temperature was kept at 27°C and the skin temperature at about 36°C. The experiment was performed at the same time of day on each occasion.

The results are summarized in Table 1. In subjects 1-5 the tyramine dose required to constrict hand vein diameter by 50% was greater when the subjects received desmethylimipramine than when they had placebo. In subject 6 not only were the two dose-response curves almost identical but there was more tyramine required following placebo than after desmethylimipramine. Taking all the results, no significant correlation was found between tyramine sensitivity and plasma concentration of desmethylimipramine. In this type of experiment a steady-state plasma level is a necessity, as the experiment takes 2 or more hours to complete. Further tachyphylaxis with tyramine was much more marked with this technique than with the pressor study probably because it was given as slow infusion. The technique is also partly dependent on the ambient temperature and emotional state of the subject.

Nevertheless, despite these drawbacks, of which the first can be eliminated by carrying out the test during steady-state plasma level, the method may have some place in subjects where large and rapid doses of tyramine are contraindicated.

It is clear from the above experiments, that the tyramine pressor response test can detect the noradrenaline blocking effect of desmethylimipramine in man 3 h after a single oral dose and may be of value in assessing potential adrenergic interactions of new, similar compounds in man.

The hand vein method may be helpful to study interactions in depressive patients suffering from cardiovascular disorder.

The rapid onset of noradrenaline reuptake blockade following a single dose of desmethylimipramine is difficult to relate to the delay in clinical response which occurs following treatment with this and similar antidepressant drugs. This suggests that the monoamine theory of affective disorders (Silverstone & Turner, 1974) may require re-evaluation in order to explain this paradox between the pharmacological and therapeutic effect of the drug.

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Table 1 The venoconstrictor effect of tyramine following placebo and desmethylimipramine (50 mg) (DMI) administration

Female subjects aged 20-24 years	*Tyramine (mg/min) required to constrict hand vein diameter by 50%*		*Tyramine sensitivity: DMI response/placebo response*	*Plasma concentration of DMI (mg/ml) at*	
	Placebo	*DMI*		*3 h*	*5 h*
1	31.1	68.7	2.2	73	35
2	72.1	164.2	2.3	<10	58
3	15.8	229.9	14.5	20	55
4	35.7	457.1	12.7	28	52
5	2.7	5.8	2.1	28	5.5
6	65.0	48.4	<1.0	25	60

was prepared by the Department of Pharmacy, St Bartholomew's Hospital.

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SENSITIVE METHOD FOR THE DETERMINATION OF TANDAMINE HYDROCHLORIDE IN PLASMA USING A SPECIFIC NITROGEN DETECTOR

Recently a new class of compounds, having a substituted thiopyrano-indole ring structure has been synthesized of which tandamine hydrochloride (Figure 1) is an example that is now being investigated.

Animal studies (Ayerst Research Laboratories) suggest that tandamine hydrochloride might have potential use as an antidepressant drug and studies of *in vivo* pharmacokinetics required a sensitive and specific method of assay. The sensitivity of the alkali flame ionization detector to the tricyclic antidepressant drugs has recently been shown to provide a suitable method for the determination of these drugs in routine plasma samples (Gifford, Turner & Pare, 1975). The method presented here, is used to estimate plasma levels of this new tricyclic antidepressant after a single oral dose.

Analyses were performed using an isothermal chromatograph with heated nitrogen detector (Perkin-Elmer, Beaconsfield, Great Britain) and

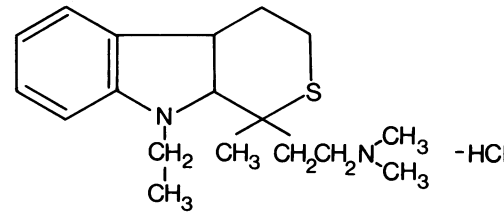

Figure 1 Structural formula of tandamine.

linear recorder. The back flush system previously reported in the study on tricyclic antidepressants was used in conjunction with a 9ft x ¼ in o.d. glass column (Gifford *et al.*, 1975). The column was silanized with a 5% solution of dimethyldichlorosilane in chloroform prior to packing with 3% OV-17 on Chromasorb W, 100-120 mesh. The packed column was conditioned at 300°C for 24 h with nitrogen